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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US99/13961 <b>(22) International Filing Date:</b> 21 June 1999 (21.06.99)  <b>(30) Priority Data:</b> <table border="0"><tr><td>60/090,497</td><td>24 June 1998 (24.06.98)</td><td>US</td></tr><tr><td>60/092,917</td><td>15 July 1998 (15.07.98)</td><td>US</td></tr><tr><td>9817170.5</td><td>6 August 1998 (06.08.98)</td><td>GB</td></tr><tr><td>9819126.5</td><td>2 September 1998 (02.09.98)</td><td>GB</td></tr></table> <b>(71) Applicant (for all designated States except US):</b> MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only):</b> RESZKA, Alfred, A. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).  <b>(74) Common Representative:</b> MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		60/090,497	24 June 1998 (24.06.98)	US	60/092,917	15 July 1998 (15.07.98)	US	9817170.5	6 August 1998 (06.08.98)	GB	9819126.5	2 September 1998 (02.09.98)	GB	<b>(81) Designated States:</b> AE, AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
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<b>(54) Title:</b> COMPOSITIONS AND METHODS FOR INHIBITING BONE RESORPTION  <b>(57) Abstract</b> <p>The present invention relates to oral compositions and methods for inhibiting bone resorption in a mammal while counteracting the occurrence of potentially adverse gastrointestinal effects. The compositions useful herein comprise the combination of a pharmaceutically effective amount of a nitrogen-containing bisphosphonate or a pharmaceutically-acceptable salt thereof and a pharmaceutically effective amount of a caspase inhibitor.</p>														

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TITLE OF THE INVENTION  
COMPOSITIONS AND METHODS FOR INHIBITING BONE RESORPTION

BRIEF DESCRIPTION OF THE INVENTION

5           The present invention relates to oral compositions and methods for  
inhibiting bone resorption in a mammal while counteracting potential adverse  
gastrointestinal effects. The compositions useful herein comprise the combination of  
a pharmaceutically effective amount of a nitrogen-containing bisphosphonate or a  
pharmaceutically-acceptable salt thereof and a pharmaceutically effective amount of a  
10   caspase inhibitor.

BACKGROUND OF THE INVENTION

          A variety of disorders in humans and other mammals involve or are  
associated with abnormal bone resorption. Such disorders include, but are not limited  
15   to, osteoporosis, Paget's disease, periprosthetic bone loss or osteolysis, and  
hypercalcemia of malignancy. The most common of these disorders is osteoporosis,  
which in its most frequent manifestation occurs in postmenopausal women.  
Osteoporosis is a systemic skeletal disease characterized by a low bone mass and  
microarchitectural deterioration of bone tissue, with a consequent increase in bone  
20   fragility and susceptibility to fracture. Osteoporotic fractures are a major cause of  
morbidity and mortality in the elderly population. As many as 70% of women and a  
third of men will experience an osteoporotic fracture. A large segment of the older  
population already has low bone density and a high risk of fractures. There is a  
significant need to both prevent and treat osteoporosis and other conditions associated  
25   with bone resorption. Because osteoporosis, as well as other disorders associated with  
bone loss, are generally chronic conditions, it is believed that appropriate therapy will  
typically require chronic treatment.

          Multinucleated cells called osteoclasts are responsible for causing  
bone loss through a process known as bone resorption. It is well known that  
30   bisphosphonates are selective inhibitors of osteoclastic bone resorption, making these  
compounds important therapeutic agents in the treatment or prevention of a variety of  
generalized or localized bone disorders caused by or associated with abnormal bone  
resorption. See H. Fleisch, *Bisphosphonates In Bone Disease, From The Laboratory  
To The Patient*, 3rd Edition, Parthenon Publishing (1997), which is incorporated by  
35   reference herein in its entirety. Without being limited by theory, it is believed that

bisphosphonates inhibit osteoclast function by triggering apoptosis, i.e. programmed cell death. See D.E. Hughes et al., "Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo", *Journal of Bone and Mineral Research*, 10 (10), 1478-1487 (1995), which is incorporated by reference herein in its entirety.

5           At present, a great amount of preclinical and clinical data exists for the potent aminobisphosphonate compound alendronate. Evidence suggests that other nitrogen-containing bisphosphonates such as pamidronate, risedronate, ibandronate and zoledronate, have many properties in common with alendronate, including high potency as inhibitors of osteoclastic bone resorption.

10           Despite their therapeutic benefits, bisphosphonates are poorly absorbed from the gastrointestinal tract. See B.J. Gertz et al., *Clinical Pharmacology of Alendronate Sodium*, *Osteoporosis Int.*, Suppl. 3: S13-16 (1993) and B.J. Gertz et al., *Studies of the oral bioavailability of alendronate*, *Clinical Pharmacology & Therapeutics*, vol. 58, number 3, pp. 288-298 (September 1995), which are  
15 incorporated by reference herein in their entirety. Intravenous administration has been used to overcome this bioavailability problem. However, intravenous administration is costly and inconvenient, especially when the patient must be given an intravenous infusion lasting several hours on repeated occasions.

          If oral administration of the bisphosphonate is desired, relatively high  
20 doses must be administered to compensate for the low bioavailability from the gastrointestinal tract. To offset this bioavailability problem, it is generally recommended that the patient take the bisphosphonate on an empty stomach and fast for at least 30 minutes afterwards. However, many patients find the need for such fasting on a daily basis to be inconvenient. Moreover, oral administration has been  
25 associated with adverse gastrointestinal effects, especially those relating to the esophagus. See Fleisch, Id. These effects appear to be related to the irritant potential of the bisphosphonate in the esophagus, a problem which is exacerbated by the presence of refluxed gastric acid. Without being limited by theory, it is believed that the potential gastrointestinal effects of oral bisphosphonates is related to the induction  
30 of apoptosis, i.e. programmed death, of the cells of the epithelial lining of the gastrointestinal tract.

          For example, the bisphosphonate, pamidronate has been associated with esophageal ulcers. See E.G. Lufkin et al., *Pamidronate: An Unrecognized Problem in Gastrointestinal Tolerability*, *Osteoporosis International*, 4: 320-322  
35 (1994), which is incorporated by reference herein in its entirety. Although not as

common, the use of alendronate has been associated with esophagitis and/or esophageal ulcers. See P.C. De Groen, et al., *Esophagitis Associated With The Use Of Alendronate*, *New England Journal of Medicine*, vol. 335, no. 124, pp. 1016-1021 (1996), D.O. Castell, *Pill Esophagitis -- The Case of Alendronate*, *New England Journal of Medicine*, vol. 335, no. 124, pp. 1058-1059 (1996), and U.A. Liberman et al., *Esophagitis and Alendronate*, *New England Journal of Medicine*, vol. 335, no. 124, pp. 1069-1070 (1996), which are incorporated by reference herein in their entirety. The degree of adverse gastrointestinal effects of bisphosphonates has been shown to increase with increasing dose. See C.H. Chestnut et al., *Alendronate Treatment of the Postmenopausal Osteoporotic Woman: Effect of Multiple Dosages on Bone Mass and Bone Remodeling*, *The American Journal of Medicine*, vol. 99, pp. 144-152, (August 1995), which is incorporated by reference herein in its entirety. Also, these adverse esophageal effects appear to be more prevalent in patients who do not take the bisphosphonate with an adequate amount of liquid or who lie down shortly after dosing, thereby increasing the chance for esophageal reflux.

Bisphosphonate treatment regimens normally involve the chronic administration of relatively low doses of the bisphosphonate compound, with the objective of delivering the desired cumulative therapeutic dose over the course of the treatment period. However, chronic dosing, especially chronic daily dosing, can have the disadvantage of causing adverse gastrointestinal effects due to the repetitive, continuous, and additive irritation to the gastrointestinal tract. This potential problem can therefore interfere with patient compliance, and in severe cases even require cessation of treatment. Therefore, it is seen that there is a need to minimize the potential adverse effects that can be associated with bisphosphonate bone resorption therapy.

Caspases belong to a broad class of enzymes known as proteases, which are enzymes that hydrolyze peptide bonds. Specifically, caspases are cysteine proteases that preferentially target and cleave peptide sequences having an aspartic acid moiety. Caspases are believed to be involved in the normal cell turnover process and are mediators of apoptosis. See Nicholson and Thornberry, 1997, "Caspases: killer proteases", *TIBS*, vol. 22, 299-306; Henkart, 1996, "ICE family proteases: mediators of all apoptotic cell death?" *Immunity*, 4, 195-201; Ray et al, 1992 "Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 beta converting enzyme", *Cell*, 69, 596-604; Enari et al, 1995, "Involvement of an ICE-like protease in fas-mediated apoptosis", *Nature*, 375, 78-81; Enari et al, 1996,

“Sequential activation of ICE-like and CPP32-like proteases during Fas-mediated apoptosis”, *Nature*, 380, 723-726; Los et al, 1995, “Requirement of an ICE/CED-3 protease for Fas/APO-1 mediated apoptosis”, *Nature*, 375, 81-83; and Tewari et al., 1995, “Fas and tumor necrosis factor-induced apoptosis is inhibited by the poxvirus crmA gene product”, *J. Biol. Chem.*, 270, 3255-3260; which are all incorporated by reference herein in their entirety.

It is found in the present invention that caspase inhibitors block the bisphosphonate-induced osteoclast apoptosis. For example, in osteoclast cell cultures and bone resorption assays, caspase inhibitors such as Z-Val-Ala-Asp-FMK, Z-Asp-Glu-Val-Asp-FMK, and Z-Tyr-Val-Ala-Asp-FMK can block the inhibitory effect that would otherwise be observed with a bisphosphonate such as alendronate monosodium trihydrate. In the osteoclasts, it is believed that nitrogen-containing bisphosphonates trigger the cleavage of a kinase known as Mst (mammalian Sterile-20-like kinase). At least two isoforms of Mst are known, i.e. “Mst 1” and “Mst 2”. See, Taylor et al., “Newly identified stress-responsive protein kinases, Krs-1 and Krs-2”, *Proc. Natl. Acad. Sci. USA*, Vol. 93 (1996), pp. 10099-10104; Creasy et al., “Cloning and characterization of a human protein kinase with homology to Ste20,” *The J. of Biological Chemistry*, Vol. 270, No. 37 (1995), pp. 21695-21700; Creasy et al., “Cloning and characterization of a member of the MST subfamily of Ste20-like kinases”, *Gene*, Vol. 167 (1995), pp. 303-306; Creasy et al., “The Ste 20-like protein kinase, Mst 1, dimerizes and contains an inhibitory domain”, *The J. of Biological Chemistry*, Vol. 271, No. 35 (1996), pp. 21049-21053; and Wang and Erikson, “Activation of protein serine/threonine kinases p42, p63, and p87 in Rous sarcoma virus-transformed cells: signal transduction/transformation-dependent MBP kinases”, *Mol. Biol. Cell*, 3, pp. 1329-1337 (1992), which are all incorporated by reference herein in their entirety. Mst cleavage is known to be triggered by cellular stress events, and is associated with apoptosis. See Graves et al., *EMBO Journal*, 17, 2224-2234, 1998, which is incorporated by reference herein in its entirety. A caspase inhibitor such as Z-VAD-FMK, however, is found to prevent the cleavage of Mst that is induced by bisphosphonates such as alendronate monosodium trihydrate. However, caspase inhibitors have not previously been investigated either *in vitro* or *in vivo* for their ability to mitigate the potentially adverse gastrointestinal side effects that can be associated with bisphosphonate anti-bone resorption therapy.

In the present invention, the combination of a nitrogen-containing bisphosphonate or a pharmaceutically-acceptable salt thereof and a caspase inhibitor



is highly effective for inhibiting bone resorption while mitigating the potentially adverse gastrointestinal effects that can be associated with bisphosphonate therapy. The combination has the advantage of providing increased safety and better patient compliance, which should maximize therapeutic efficacy. Without being limited by theory it is believed that the caspase inhibitor blocks the potentially harmful effect of the bisphosphonate on the epithelial cells of the gastrointestinal tract. By selecting an appropriate dosage of the caspase inhibitor it is possible to orally deliver a sufficiently high local concentration of the caspase inhibitor to the gastrointestinal tract to block the potentially harmful effects of the nitrogen-containing bisphosphonate, while minimizing the blocking effect on the osteoclasts, where the full therapeutic benefit of the bisphosphonate is desired to inhibit bone resorption.

It is an object of the present invention to provide compositions comprising the combination of a nitrogen-containing bisphosphonate or a pharmaceutically-acceptable salt thereof and a caspase inhibitor.

It is another object of the present invention to provide improved oral methods for inhibiting bone resorption and the conditions associated therewith in a mammal, particularly wherein said mammal is a human.

It is another object of the present invention to provide improved oral methods for treating or preventing abnormal bone resorption and the conditions associated therewith.

It is another object of the present invention to provide such oral methods while counteracting potential adverse gastrointestinal effects.

It is another object of the present invention to provide such methods wherein the dosing is maintained until the desired therapeutic effect is achieved.

It is another object of the present invention to treat or prevent abnormal bone resorption in an osteoporotic mammal, preferably an osteoporotic human.

These and other objects will become readily apparent from the detailed description which follows.

## SUMMARY OF THE INVENTION

The present invention relates to a pharmaceutical composition comprising a nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof and a caspase inhibitor.

In further embodiments, the present invention relates to a pharmaceutical composition comprising a pharmaceutically-effective amount of a

nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof and an amount of a caspase inhibitor effective to counteract nitrogen-containing bisphosphonate-associated gastrointestinal effects.

5 In further embodiments, the present invention relates to a method for inhibiting bone resorption in a mammal in need thereof comprising administering a nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof and a caspase inhibitor.

10 In further embodiments, the present invention relates to a method for inhibiting bone resorption in a mammal in need thereof comprising sequentially administering a caspase inhibitor and a nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof.

15 In further embodiments, the present invention relates to the use of a composition in the manufacture of a medicament for inhibiting bone resorption in a mammal in need thereof, said composition comprising a nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof and a caspase inhibitor.

In further embodiments, the present invention relates to the use of a composition comprising a nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof and a caspase inhibitor for inhibiting bone resorption in a mammal in need thereof.

20 All percentages and ratios used herein, unless otherwise indicated, are by weight. The invention hereof can comprise, consist of, or consist essentially of the essential as well as optional ingredients, components, and methods described herein.

#### BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the identification of a 34 kDa Mst kinase fragment that is activated by 30  $\mu$ M alendronate monosodium trihydrate (a nitrogen-containing bisphosphonate). The 34 kDa Mst kinase fragment is an amino-terminal cleavage product of Mst. Osteoclast-like cells are isolated and treated with alendronate monosodium trihydrate. Cell lysates are exposed to treatment with a cocktail of  
30 commercially available anti-Mst and anti-Krs antibodies that recognize the amino-terminus of Mst kinase and Protein A-agarose beads to immunoprecipitate Mst from the samples. The samples are analyzed using an in-gel kinase assay. These data demonstrate that the anti-Mst and anti-Krs antibody cocktail immunoprecipitates 59 and 60 kDa Mst Kinases as well as a 34 kDa Mst Kinase fragment in untreated  
35 osteoclasts (lane 2) and alendronate monosodium trihydrate-treated osteoclasts (lane

3). No kinase activities are immunoprecipitated in the absence of anti-Mst and anti-Krs antibodies (lane 1). These data also demonstrate that the 34 kDa Mst Kinase fragment immunoprecipitated by the anti-Mst and anti-Krs antibody cocktail is activated by alendronate monosodium trihydrate (lane 3).

5                   Figure 2 shows the preferential response of a 34 kDa Mst Kinase fragment to alendronate monosodium trihydrate (a nitrogen-containing bisphosphonate) versus etidronate disodium or tiludronate disodium (both of which are non-nitrogen-containing bisphosphonates). Osteoclast like cells are treated with alendronate monosodium trihydrate, etidronate disodium, or tiludronate disodium at  
10 concentrations of both 30  $\mu$ M and 100  $\mu$ M for about 17 hours. Kinase activities are analyzed using the in-gel kinase assays. Autoradiographs are developed after film exposure for several days. These data show the activation of the 34 kDa Mst Kinase fragment by alendronate monosodium trihydrate at either 30  $\mu$ M (lane 3) or 100  $\mu$ M (lane 4). Equivalent doses of etidronate disodium (lanes 5 and 6) or tiludronate  
15 disodium (lanes 7 and 8), fail to activate this kinase fragment, with results being similar to no treatment controls (lanes 1 and 2).

                  Figure 3 shows the preferential response of 34 kDa Kinase to alendronate monosodium trihydrate and the inability of alendronate monosodium trihydrate to activate the 34 kDa Kinase when used to treat osteoclasts in the presence  
20 of the caspase inhibitor Z-Val-Ala-Asp-FMK. Osteoclast-like cells are treated with alendronate monosodium trihydrate at concentrations of 10  $\mu$ M and 30  $\mu$ M for about 17 hours. Kinase activities are analyzed using an in-gel kinase assay. Autoradiographs are developed after film exposure for several days. These data show the activation of a 34 kDa Kinase by alendronate at either 10  $\mu$ M (lane 2) or 30  $\mu$ M  
25 (lane 3). Alendronate does not activate the 34 kDa Kinase when coincubated in the presence of Z-Val-Ala-Asp-FMK (lane 8). Higher doses of etidronate disodium, i.e. 100  $\mu$ M (lane 4) and 300  $\mu$ M (lane 5), or tiludronate disodium, i.e. 100  $\mu$ M (lane 6) and 300  $\mu$ M (lane 7), fail to activate this kinase after 17 hours of treatment and are similar to no treatment control (lane 1). See J.D. Graves et al., "Caspase-mediated  
30 activation and induction of apoptosis by the mammalian Ste2-like kinase Mst1", The EMBO Journal, vol. 17, no. 8, pp. 2224-2234 (1998), which has already been incorporated by reference herein in its entirety.

                  Figure 4 shows that activation of Mst 1 by 30  $\mu$ M alendronate monosodium trihydrate is blocked by caspase inhibitors. Osteoclast-like-cells are  
35 purified from cocultures by sequential treatment of culture dishes with collagenase and

EDTA. After the desired treatment, cell lysates are analyzed by an in-gel kinase assay using myelin basic protein as a substrate. Lane 1 is a no-treatment control. Lane 2 shows treatment with 20  $\mu$ M Z-Asp-Glu-Val-Asp-FMK. Lane 3 shows treatment with 20  $\mu$ M Z-Val-Ala-Asp-FMK. Lane 4 shows treatment with 20  $\mu$ M Z-Tyr-Val-Ala-Asp-FMK. Lane 5 shows treatment with 30  $\mu$ M alendronate monosodium trihydrate. Lane 6 shows treatment with 30  $\mu$ M alendronate monosodium trihydrate and 20  $\mu$ M Z-Asp-Glu-Val-Asp-FMK. Lane 7 shows treatment with 30  $\mu$ M alendronate monosodium trihydrate and 20  $\mu$ M Z-Val-Ala-Asp-FMK. Lane 8 shows treatment with 30  $\mu$ M alendronate monosodium trihydrate and 20  $\mu$ M Z-Tyr-Val-Ala-Asp-FMK.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compositions and methods for inhibiting bone resorption in a mammal in need of such treatment, while counteracting the occurrence of adverse gastrointestinal effects. The compositions comprise a pharmaceutically effective amount of a nitrogen-containing bisphosphonate or a pharmaceutically-acceptable salt thereof and a pharmaceutically effective amount of a caspase inhibitor.

The term "pharmaceutically effective amount", as used herein, means that amount of the nitrogen-containing bisphosphonate compound or caspase inhibitor, that will elicit the desired therapeutic effect or response or provide the desired benefit when administered in accordance with the desired treatment regimen. A preferred pharmaceutically effective amount of the nitrogen-containing bisphosphonate is a bone resorption inhibiting amount. A preferred pharmaceutically effective amount of the caspase inhibitor is an amount that will counteract, i.e. block or mitigate, the occurrence of adverse gastrointestinal effects, while not counteracting, or only minimally counteracting, the therapeutic bone resorption effects of the nitrogen-containing bisphosphonate.

The term "counteracting the occurrence of adverse gastrointestinal effects", as used herein, means to prevent, block, decrease, or lessen the occurrence of unwanted side effects in the gastrointestinal tract, i.e. the esophagus, stomach, intestines, and rectum, particularly the esophagus, relative to treatment with a nitrogen-containing bisphosphonate alone. Nonlimiting examples of adverse gastrointestinal effects include, but are not limited to those selected from the group

consisting of esophagitis, esophageal ulcers, esophageal irritation, esophageal perforation, abdominal pain, gastric duodenal ulcers, and constipation.

In the present invention it is an object to inhibit bone resorption, or more specifically to inhibit undesired or abnormal bone resorption. The term  
5 "abnormal bone resorption", as used herein means a degree of bone resorption that exceeds the degree of bone formation, either locally, or in the skeleton as a whole. Alternatively, "abnormal bone resorption" can be associated with the formation of bone having an abnormal structure, as in Paget's disease.

The term "bone resorption inhibiting", as used herein, means  
10 preventing bone resorption by the direct or indirect alteration of osteoclast formation or activity. Inhibition of bone resorption refers to prevention of bone loss, especially the inhibition of removal of existing bone either from the mineral phase and/or the organic matrix phase, through direct or indirect alteration of osteoclast formation or activity.

15 The term "until the desired therapeutic effect is achieved", as used herein, means that the therapeutic agent or agents are continuously administered, according to the dosing schedule chosen, up to the time that the clinical or medical effect sought for the disease or condition being treated is observed by the clinician or researcher. For methods of treatment of the present invention, the pharmaceutical  
20 composition is continuously administered until the desired change in bone mass or structure is observed. In such instances, achieving an increase in bone mass or a replacement of abnormal bone structure with normal bone structure are the desired objectives. For methods of prevention of the present invention, the pharmaceutical composition is continuously administered for as long as necessary to prevent the  
25 undesired condition. In such instances, maintenance of bone mass density is often the objective. Nonlimiting examples of administration periods can range from about 2 weeks to the remaining lifespan of the mammal. For humans, administration periods can range from about 2 weeks to the remaining lifespan of the human, preferably from about 2 weeks to about 20 years, more preferably from about 1 month to about 20  
30 years, more preferably from about 6 months to about 10 years, and most preferably from about 1 year to about 10 years.

The term "nitrogen-containing" as used herein means that the bisphosphonate compound or pharmaceutically acceptable salt thereof comprises at least one nitrogen atom in the bisphosphonate portion of the molecule. In other  
35 words, for a pharmaceutically-acceptable salt of the bisphosphonate, any nitrogen

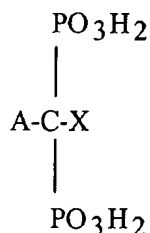
atom contained in the positive counter ion of such a salt, e.g., the nitrogen atom of an ammonium counter ion, would not be considered in meeting the "nitrogen-containing" definition. For example, alendronic acid, i.e. 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid is an example of a nitrogen-containing bisphosphonate. However, the ammonium salt of the unsubstituted 1-hydroxybutylidene-1,1-bisphosphonic acid would not be a nitrogen-containing bisphosphonate as defined herein.

#### Compositions of the present invention

The pharmaceutical compositions of the present invention comprise a pharmaceutically effective amount of a nitrogen-containing bisphosphonate or a pharmaceutically-acceptable salt thereof and a pharmaceutically effective amount of a caspase inhibitor. These compositions are useful for inhibiting bone resorption in a mammal in need thereof while counteracting the potentially adverse effects, such as gastrointestinal effects, that can be associated with the administration of the bisphosphonate.

#### Nitrogen-Containing Bisphosphonates

The nitrogen-containing bisphosphonates useful herein correspond to the chemical formula



wherein

A and X are independently selected from the group consisting of H, OH, OR, halogen, NH<sub>2</sub>, NHR, NR<sub>2</sub>, SR, SH, C1-C30 alkyl (including straight, branched, and cyclic alkyl), substituted C1-C30 alkyl (including straight, branched, and cyclic alkyl), and C5-C14 aryl, wherein said substituted C1-C30 alkyl comprises one or more moieties selected from the group consisting of OH, OR, halogen, NH<sub>2</sub>, NHR, NR<sub>2</sub>, SH, SR, and C5-C14 aryl, wherein R is selected from the group consisting of C1-C30 alkyl (including straight, branched, and cyclic alkyl) and C5-

C14 aryl, with the proviso that the resulting bisphosphonate compound contains at least one nitrogen atom. In the bisphosphonate compounds of the present invention, A and X can be taken together to form a cyclic moiety. It is also intended that any of the alkyl groups can be unsaturated with a double or triple bond at one or more positions.

Nonlimiting examples of the substituents for the substituted C1-C30 alkyl moiety include those selected from the group consisting of amino, alkylamino, dialkylamino, imidazolyl, pyridyl, imidazonyl, imidazopyridinyl, furanyl, and cycloalkylamino.

The term "aryl," as used herein, refers to a monocyclic or polycyclic system comprising at least one aromatic ring, wherein the monocyclic or polycyclic system contains 0, 1, 2, 3, or 4 heteroatoms chosen from N, O, or S, and wherein the monocyclic or polycyclic system is either unsubstituted or substituted with one or more groups independently selected from hydrogen, halogen, C1-10 alkyl, C3-8 cycloalkyl, aryl, aryl C1-8 alkyl, amino, amino C1-8 alkyl, C1-3 acylamino, C1-3 acylamino C1-8 alkyl, C1-6 alkylamino, C1-6 alkylamino C1-8 alkyl, C1-6 dialkylamino, C1-6 dialkylamino-C1-8 alkyl, C1-4 alkoxy, C1-4 alkoxy C1-6 alkyl, hydroxycarbonyl, hydroxycarbonyl C1-6 alkyl, C1-5 alkoxycarbonyl, C1-3 alkoxycarbonyl C1-6 alkyl, hydroxycarbonyl C1-6 alkyloxy, hydroxy, hydroxy C1-6 alkyl, cyano, trifluoromethyl, oxo or C1-5 alkylcarbonyloxy. Examples of aryl include, but are not limited to, phenyl, naphthyl, pyridyl, pyrazinyl, pyrimidinyl, imidazolyl, benzimidazolyl, indolyl, thienyl, furyl, dihydrobenzofuryl, benzo(1,3)dioxolane, oxazolyl, isoxazolyl and thiazolyl.

Preferred nitrogen-containing bisphosphonates are those in which A is selected from the group consisting of H, OH, and halogen, more preferably OH, and X is selected from the group consisting of NH<sub>2</sub>, NHR, NR<sub>2</sub>, C5-C14 aryl, substituted C1-C30 alkyl (including straight, branched, and cyclic alkyl) wherein said substituted alkyl group is substituted with one or more moieties selected from the group consisting of OH, OR, halogen, NH<sub>2</sub>, NHR, NR<sub>2</sub>, SH, SR, and C5-C14 aryl, with the proviso that the resulting compound contains at least one nitrogen atom.

Most preferred is when A is OH and X is a 3-aminopropyl moiety, so that the resulting compound is a 4-amino-1-hydroxybutylidene-1,1-bisphosphonate, i.e. alendronate.

Pharmaceutically acceptable salts and derivatives of the nitrogen-containing bisphosphonates are also useful herein. Nonlimiting examples of salts

include those selected from the group consisting of alkali metal, alkaline metal, ammonium, and mono-, di, tri-, or tetra-C1-C30-alkyl-substituted ammonium. Preferred salts are those selected from the group consisting of sodium, potassium, calcium, magnesium, and ammonium salts. Typically, the salts are formed when one or more of the hydrogen atoms of the phosphonate groups are substituted.

Nonlimiting examples of derivatives include those selected from the group consisting of esters and amides.

Various hydrated and anhydrous forms of the nitrogen-containing bisphosphonates are intended as within the scope of the present invention.

"Pharmaceutically-acceptable" as used herein means that the salts and derivatives of the nitrogen-containing bisphosphonates have the same general pharmacological properties as the free acid form from which they are derived and are acceptable from a toxicity standpoint.

It should be noted that the terms "bisphosphonate" and "bisphosphonates", as used herein in referring to the nitrogen-containing bisphosphonates are meant to also encompass the terms diphosphonates, bisphosphonic acids, and diphosphonic acids, as well as salts and derivatives of these materials. The use of a specific nomenclature in referring to the bisphosphonate or bisphosphonates is not meant to limit the scope of the present invention, unless specifically indicated.

Because of the mixed nomenclature currently in use by those of ordinary skill in the art, reference to a specific weight or percentage of a bisphosphonate compound in the present invention is on an acid active weight basis, unless indicated otherwise herein. For example, the phrase "about 10 mg of a nitrogen-containing bisphosphonate selected from the group consisting of alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof, on an alendronic acid active weight basis" means that the amount of the bisphosphonate compound selected is calculated based on 10 mg of alendronic acid.

Nonlimiting examples of nitrogen-containing bisphosphonates useful herein include the following:

Alendronic acid, 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid.

Alendronate (also known as alendronate sodium or monosodium trihydrate), 4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid monosodium trihydrate.



Alendronic acid and alendronate are described in U.S. Patents 4,922,007, to Kieczkowski et al., issued May 1, 1990, and 5,019,651, to Kieczkowski, issued May 28, 1991, both of which are incorporated by reference herein in their entirety.

5                   Cycloheptylaminomethylene-1,1-bisphosphonic acid, YM 175, Yamanouchi (cimadronate), as described in U.S. Patent 4,970,335, to Isomura et al., issued November 13, 1990, which is incorporated by reference herein in its entirety.

10                   1-hydroxy-3-(1-pyrrolidiny)-propylidene-1,1-bisphosphonic acid (EB-1053).

                  1-hydroxy-3-(N-methyl-N-pentylamino)propylidene-1,1-bisphosphonic acid, also known as BM-210955, Boehringer-Mannheim (ibandronate), is described in U.S. Patent No. 4,927,814, issued May 22, 1990, which is incorporated by reference herein in its entirety.

15                   6-amino-1-hydroxyhexylidene-1,1-bisphosphonic acid (neridronate).

                  3-(dimethylamino)-1-hydroxypropylidene-1,1-bisphosphonic acid (olpadronate).

20                   3-amino-1-hydroxypropylidene-1,1-bisphosphonic acid (pamidronate).

                  [2-(2-pyridinyl)ethylidene]-1,1-bisphosphonic acid (piridronate) is described in U.S. Patent No. 4,761,406, which is incorporated by reference in its entirety.

25                   1-hydroxy-2-(3-pyridinyl)-ethylidene-1,1-bisphosphonic acid (risedronate).

                  1-hydroxy-2-(1H-imidazol-1-yl)ethylidene-1,1-bisphosphonic acid (zolendronate).

                  [1-hydroxy-2-imidazo-(1,2-a)pyridin-3-ylethylidene]bis-phosphonate (Yamanouchi YH 529).

30                   Preferred are bisphosphonates selected from the group consisting of alendronate, cimadronate, ibandronate, risedronate, piridronate, pamidronate, zolendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

                  More preferred is alendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

35                   Most preferred is alendronate monosodium trihydrate.

It is recognized that mixtures of two or more of the nitrogen-containing bisphosphonates can be utilized.

The precise dosage of the nitrogen-containing bisphosphonate will vary with the dosing schedule, the particular bisphosphonate chosen, the age, size, sex and condition of the mammal or human, the nature and severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise pharmaceutically effective amount cannot be specified in advance and can be readily determined by the caregiver or clinician. Appropriate amounts can be determined by routine experimentation from animal models and human clinical studies. Generally, an appropriate amount of nitrogen-containing bisphosphonate is chosen to obtain a bone resorption inhibiting effect, i.e. a bone resorption inhibiting amount of the nitrogen-containing bisphosphonate is administered. For humans, an effective oral dose of nitrogen-containing bisphosphonate is typically from about 1.5 to about 6000  $\mu\text{g/kg}$  body weight and preferably about 10 to about 2000  $\mu\text{g/kg}$  of body weight.

For the nitrogen-containing bisphosphonate, alendronate monosodium trihydrate, common human doses which are administered are generally in the range of about 2 mg/day to about 40 mg/day, preferably about 5 mg/day to about 40 mg/day. In the U.S. presently approved dosages for alendronate monosodium trihydrate are 5 mg/day for preventing osteoporosis, 10 mg/day for treating osteoporosis, and 40 mg/day for treating Paget's disease.

In alternative dosing regimens, the nitrogen-containing bisphosphonate can be administered at intervals other than daily, for example once-weekly dosing, twice-weekly dosing, biweekly dosing, and twice-monthly dosing. In such dosing regimens, appropriate multiples of the bisphosphonate dosage would be administered. For example, in a once weekly dosing regimen, alendronate monosodium trihydrate would be administered at dosages of 35 mg/week or 70 mg/week in lieu of seven consecutive daily dosages of 5 mg or 10 mg.

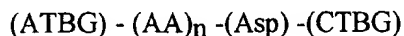
The pharmaceutical compositions herein comprise from about 1 mg to about 100 mg of nitrogen-containing bisphosphonate, preferably from about 2 mg to 70 mg, and more preferably from about 5 mg to about 70, on a bisphosphonic acid basis. For the bisphosphonate alendronate monosodium trihydrate, the pharmaceutical compositions useful herein comprise about 2.5 mg, 5 mg, 10 mg, 35 mg, 40 mg, or 70 mg of the active on an alendronic acid active weight basis.

### Caspase Inhibitors

The compositions of the present invention comprise a pharmaceutically effective amount of a caspase inhibitor.

The caspase inhibitors useful herein are generally relatively short aspartic acid-containing peptides, although non-peptide inhibitors are also intended as being within the scope of the present invention. By "relatively short", as used herein means that the peptides typically contain from about 3 to about 5 amino acids in length. By "aspartic acid-containing" is meant that these peptides comprise at least one aspartic acid moiety, preferably at the carboxy-terminal end. These peptides are preferably blocked at both the amino and carboxy terminal ends with blocking groups.

The caspase inhibitors useful herein can be represented by the following chemical formula



Wherein (ATBG) is an amino terminal blocking group, (AA) is an amino acid moiety, "Asp" is aspartic acid moiety, (CTBG) is a carboxy terminal blocking group, and n is an integer from about 2 to about 4. In the caspase inhibitors, the amino acid (AA) can be selected from any of the naturally occurring amino acids, the D-enantiomers of the naturally-occurring amino acids (for example, D-alanine), and non-naturally occurring amino acids (for example, 3-aminopropionic acid and N-methyl glycine). The (ATBG) and (CTBG) moieties are selected from any of the blocking groups that are well known to peptide chemists of ordinary skill in the art. *See* Greene, T.W. et al., *Protecting Groups in Organic Synthesis*, 2nd edition, 1991, John Wiley & Sons, Inc., which is incorporated by reference herein in its entirety. Nonlimiting examples of (ATBG) moieties are benzyloxycarbonyl group (also known as the cbz or Z group) and the *t*-butoxycarbonyl group (also known as the boc group), and the acyl group. Nonlimiting examples of (CTBG) moieties are alkyl groups (for example methyl and ethyl esters), the benzyl group, and the fluoromethyl keto group [which is abbreviated as (OMe)-CH<sub>2</sub>F or FMK].

Nonlimiting examples of caspase inhibitors useful herein are disclosed in U.S. Patent No. 5,210, 272, to Palmer, issued May 11, 1993, U.S. Patent 5,101,068, to Palmer et al., issued March 31, 1992, and U.S. Patent No. 4,518,528, to Rasnick, issued May 21, 1985, which are all incorporated by reference herein in their entirety. Preferred caspase inhibitors useful herein are selected from the group consisting of Z-Val-Ala-Asp-FMK (which has a molecular weight of about 468), Z-Asp-Glu-Val-

Asp-FMK (which has a molecular weight of about 668), and Z-Tyr-Val- Ala-Asp-FMK (which has a molecular weight of about 630) MW 630. In the foregoing the standard three-letter amino acid abbreviations were used.

5 It is recognized that mixtures of two or more of the caspase inhibitors can be utilized.

The precise dosage of the caspase inhibitor will vary with the dosing schedule, the particular compound chosen, the age, size, sex and condition of the mammal or human, the nature and severity of the disorder to be treated, and other relevant medical and physical factors. Thus, a precise pharmaceutically effective  
10 amount cannot be specified in advance and can be readily determined by the caregiver or clinician. Appropriate amounts can be determined by routine experimentation from animal models and human clinical studies. Generally, an appropriate amount is chosen to obtain an inhibition of the potentially adverse gastrointestinal effects of the nitrogen-containing bisphosphonate. The amount should be below that level which  
15 will inhibit the desired bone resorption inhibiting effect of the nitrogen-containing bisphosphonate. For humans, an effective oral dose of the caspase inhibitor is typically chosen so as to provide a local concentration in the esophagus from about 1  $\mu$ M to about 100  $\mu$ M, preferably about 10  $\mu$ M, although other ranges can be used. Nonlimiting exemplary doses are about 1 ug/kg to about 100 ug/kg, preferably about  
20 10 ug/kg, for a human subject.

For the caspase inhibitor, human doses which can be administered are generally in the range of about 0.1 mg/day to about 10 mg/day, preferably from about 0.25 mg/day to about 5 mg/day, and more preferably from about 0.5 mg/day to about 1.5 mg/day. A typical nonlimiting dosage amount would be about 0.75 mg/day. The  
25 pharmaceutical compositions herein comprise from about 0.1 mg to about 10 mg, preferably from about 0.25 mg to about 5 mg, and more preferably from about 0.5 mg to about 1.5 mg of the caspase inhibitor. A typical nonlimiting amount for is about 0.75 mg.

### 30 Other components of the pharmaceutical compositions

The nitrogen-containing bisphosphonate and the caspase inhibitor are typically administered in admixture with suitable pharmaceutical diluents, excipients, or carriers, collectively referred to herein as "carrier materials", suitably selected with respect to oral administration, i.e. tablets, capsules, elixirs, syrups, powders, and the  
35 like, and consistent with conventional pharmaceutical practices. For example, for oral

administration in the form of a tablet, capsule, or powder, the active ingredient can be combined with an oral, non-toxic, pharmaceutically acceptable inert carrier such as lactose, starch, sucrose, glucose, methyl cellulose, magnesium stearate, mannitol, sorbitol, croscarmellose sodium and the like; for oral administration in liquid form, e.g., elixirs and syrups, the oral drug components can be combined with any oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated. Suitable binders can include starch, gelatin, natural sugars such as glucose, anhydrous lactose, free-flow lactose, beta-lactose, and corn sweeteners, natural and synthetic gums, such as acacia, guar, tragacanth or sodium alginate, carboxymethyl cellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride and the like. A particularly preferred tablet formulation for alendronate monosodium trihydrate is that described in U.S. Patent No. 5,358,941, to Bechard et al, issued October 25, 1994, which is incorporated by reference herein in its entirety. The compounds used in the present method can also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxylpropyl-methacrylamide, and the like.

#### Methods of the Present Invention

The present invention comprises methods for treating abnormal bone resorption in mammals. The present invention also comprises methods for preventing abnormal bone resorption in mammals. In preferred embodiments of the present invention, the mammal is a human.

The methods and compositions of the present invention are useful for both treating and preventing abnormal bone resorption and conditions associated therewith. Conditions associated with abnormal bone resorption include both generalized and localized bone loss. Also, the creation of bone having an abnormal structure, as in Paget's disease, can be associated with abnormal bone resorption. The term "generalized bone loss" means bone loss at multiple skeletal sites or throughout the skeletal system. The term "localized bone loss" means bone loss at one or more specific, defined skeletal sites.

Generalized bone loss is often associated with osteoporosis. Osteoporosis is most common in post-menopausal women, wherein estrogen

production has been greatly diminished. However, osteoporosis can also be steroid-induced and has been observed in males due to age. Osteoporosis can be induced by disease, e.g. rheumatoid arthritis, it can be induced by secondary causes, e.g., glucocorticoid therapy, or it can come about with no identifiable cause, i.e. idiopathic osteoporosis. In the present invention, preferred methods include the treatment or prevention of abnormal bone resorption in osteoporotic humans.

Localized bone loss has been associated with periodontal disease, with bone fractures, and with periprosthetic osteolysis (in other words where bone resorption has occurred in proximity to a prosthetic implant).

Generalized or localized bone loss can occur from disuse, which is often a problem for those confined to a bed or a wheelchair, or for those who have an immobilized limb set in a cast or in traction.

The methods and compositions of the present invention are useful for treating and or preventing the following conditions or disease states: osteoporosis, which can include post-menopausal osteoporosis, steroid-induced osteoporosis, male osteoporosis, disease-induced osteoporosis, idiopathic osteoporosis; Paget's disease; abnormally increased bone turnover; osteomalacia; periodontal disease; localized bone loss associated with periprosthetic osteolysis; and bone fractures.

The compositions and methods of the present invention are administered and carried out until the desired therapeutic effect is achieved.

In the methods of the present invention the nitrogen-containing bisphosphonate and the caspase inhibitor are generally administered concurrently. In alternate embodiments, the nitrogen-containing bisphosphonate and the caspase inhibitor can be administered sequentially. Preferably, the caspase inhibitor is administered first.

The following Examples are presented to better illustrate the invention.

#### EXAMPLE 1

##### Method for Evaluating the Effect of a Nitrogen-Containing Bisphosphonate and a Caspase Inhibitor on Kinase Activities in Cultured Osteoclasts

Murine co-cultures of osteoblasts and marrow cells are prepared using the methods of Wesolowski, et al., *Exp Cell Res*, (1995), 219, pp. 679-686, which is incorporated by reference herein in its entirety. Bone marrow cells are harvested from 6-week-old male Balb/C mice by flushing marrow spaces of freshly isolated long

bones (tibiae and femora) with  $\alpha$ -MEM (minimal essential media) containing penicillin/streptomycin (100 I.U./ml of each and 20 mM Hepes buffer). The bone marrow cells are suspended in  $\alpha$ -MEM and the cells are filtered through an approximately 70  $\mu$ m cell strainer. The filtrate is centrifuged at about 300 x g for about 7 minutes. The resulting pellet is resuspended in  $\alpha$ -MEM supplemented with fetal calf serum (10 % v/v) and 10 nM 1, 25-(OH)<sub>2</sub> vitamin D<sub>3</sub>. These bone marrow isolates are added to sub-confluent monolayers of osteoblastic MB 1.8 cells in cell culture plates and cultured for 7 days at 37°C in the presence of 5% CO<sub>2</sub>. Culture media is replenished ever other day. Fusion of the osteoclast precursor cells from bone marrow (with each other) to form multinucleated osteoclast-like cells typically occurs after about 7 days. Osteoclast-like cells are enriched by sequential treatment with collagenase (1 mg/mL in phosphate buffered saline) for one hour at 37°C and EDTA (0.2 g/L in phosphate buffered saline) for 20 min at 37°C. Non-adherent cells are rinsed away by washing with phosphate buffered saline. Osteoclast-like cells which are resistant to the sequential treatments are present at about 95% purity and are maintained in  $\alpha$ -MEM supplemented with fetal calf serum (10 % v/v), 10 nM 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub>, macrophage-colony-stimulating factor (5 ng/mL).

The compounds to be evaluated are prepared as a solution of the desired concentration in  $\alpha$ -MEM. Examples of compounds that can be evaluated include nitrogen-containing bisphosphonates such as alendronate monosodium trihydrate, as well as compounds that block the effects of these inhibitors, such as caspase inhibitors such as Z-Val-Ala-Asp-FMK, Z-Asp-Glu-Val-Asp-FMK, and Z-Tyr-Val-Ala-Asp-FMK. Combinations of compounds can also be evaluated. The solutions of the compounds to be evaluated are added to the cultures for a time period of 17-24 hours. No treatment controls (controls not treated with compounds) are prepared by adding equivalent volumes of  $\alpha$ -MEM to the control dishes.

Cells are then harvested and lysed in a HEPES (N-(2-hydroxyethyl)piperazine-N'-(2-ethansulfonic acid) or Tris buffer containing the following:  $\beta$ -glycerophosphate (50 mM); Na<sub>3</sub>VO<sub>4</sub> (1mM); NaF (1mM); Microcystin LR (1  $\mu$ M); leupeptin (10  $\mu$ g/ml); aprotinin (10  $\mu$ g/ml); phenylmethyl sulfonylfluoride (1 mM). Protein concentrations are determined for each lysate and 5-20  $\mu$ g are loaded into each lane of a SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gel containing Myelin Basic Protein, or another kinase substrate, which has been polymerized into the gel at a concentration between 50-400  $\mu$ g/ml.

Molecular weight standards are also loaded into one or more lanes of the gels. In-gel kinase assays are run according to a standard procedure based on Kameshita and Fujisawa, 1989 (Anal. Biochem. 183:139-143) and of Gotoh et al., 1990 (Eur. J. Biochem. 193: 661-669), both references being incorporated by reference herein in their entirety. The proteins are electrophoresed in the above gels. The gels are then successively soaked in 50 mM HEPES, pH 7.6; 5 mM 2-mercaptoethanol and each of the following (for each wash): (a) 20% isopropanol; (b) no additions; (c) urea (6 M); (d) Urea (3 M); (e) Urea (0.75 M); and Tween 20 (0.05% vol:vol). Kinase reactions are then run by first soaking the gels in 20 mM HEPES, pH 7.6; 20 mM MgCl<sub>2</sub>; 2 mM DTT and then in the same buffer containing 0.02 M ATP (non-radioactive) with ca. 1000 cpm/pmol <sup>32</sup>P-γ-ATP. The gels are then washed six times with 5% trichloroacetic acid and 1% pyrophosphate. The gels are then stained with Coomassie brilliant blue dye (0.125%) in 50% methanol, 10% acetic acid; destained with 30% methanol, 10% acetic acid; soaked in 2% glycerol; and dried using a gel dryer. The gels are then exposed to autoradiography film for times ranging from several hours to weeks. The bands observed in the autoradiographs representing the gels reflect kinase activities. Mst 1 (apparent molecular weight about 59 kDa), Mst 2 (apparent molecular weight about 60 kDa), and a 34 kDa Mst kinase fragment are observed and identified by their migration as compared to the migration of molecular weight standards. The band intensities on the autoradiography film are quantitated by densitometry and comparisons between bands from untreated controls and bands from echistatin-treated cells provide the basis for the analyses.

## EXAMPLE 2

### Pharmaceutical Tablet Compositions

Tablets are prepared using standard mixing and formation techniques as described in U.S. Patent No. 5,358,941, to Bechard et al., issued October 25, 1994, which is incorporated by reference herein in its entirety.

Tablets containing about 10 mg of alendronate monosodium trihydrate, on an alendronic acid active basis, and about 0.75 mg of Z-VAD-FMK, are prepared using the following relative weights of ingredients.



	<u>Ingredient</u>	<u>Per Tablet</u>	<u>Per 4000 Tablets</u>
	Alendronate Monosodium	13.051 mg	52.20 g
5	Trihydrate		
	Z-Val-Ala-Asp-FMK	0.75 mg	3.00 g
	Anhydrous Lactose, NF	71.32 mg	285.28 g
	Microcrystalline Cellulose, NF	80.0 mg	320.0 g
	Magnesium Stearate, NF	1.0 mg	4.0 g
10	Croscarmellose Sodium, NF	2.0 mg	8.0 g

The resulting tablets are useful for administration in accordance with the methods of the present invention for treating or preventing bone resorption.

Similarly, tablets comprising other relative weights of alendronate, on  
 15 an alendronic acid active weight basis are prepared. Also, tablets containing other nitrogen-containing bisphosphonates, or mixtures thereof, at appropriate active levels are similarly prepared: e.g., cimadronate, ibandronate, risedronate, piridronate, pamidronate, zolendronate, and pharmaceutically acceptable salts thereof. Also, tablets containing combinations of bisphosphonates are similarly prepared.

20 Similarly, tablets comprising other caspase inhibitors such as Z-Asp-Glu-Val- Asp-FMK or Z-Tyr-Val- Ala-Asp-FMK are prepared.

The tablets described herein are useful for inhibiting bone resorption while mitigating potentially adverse gastrointestinal effects.

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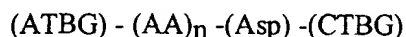
## WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a nitrogen-  
containing bisphosphonate or a pharmaceutically acceptable salt thereof and a caspase  
5 inhibitor.

2. A pharmaceutical composition according to Claim 1 wherein  
said nitrogen containing bisphosphonate is selected from the group consisting of  
alendronate, cimidronate, ibandronate, risedronate, piridronate, pamidronate,  
10 zolendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.

3. A pharmaceutical composition according to Claim 2 wherein  
said caspase inhibitor is an aspartic acid-containing caspase inhibitor.

4, A pharmaceutical composition according to Claim 2 wherein  
15 said caspase inhibitor corresponds to the following chemical formula



20 wherein (ATBG) is an amino terminal blocking group selected from the group  
consisting of benzyloxycarbonyl, t-butoxycarbonyl, and acyl, (AA) is an amino acid,  
(Asp) is aspartic acid, (CBTG) is a carboxy terminal blocking group selected from the  
group consisting of C<sub>1</sub>-C<sub>6</sub> alkyl, benzyl, and fluoromethylketo, and n is an integer  
from about 2 to about 4.

25

5. A pharmaceutical composition according to Claim 4 wherein  
said caspase inhibitor is selected from the group consisting of Z-Val-Ala-Asp-FMK,  
Z-Asp-Glu-Val-Asp-FMK, Z-Tyr-Val-Ala-Asp-FMK, and mixtures thereof.

30

6. A pharmaceutical composition according to Claim 5 wherein  
said caspase inhibitor is Z-Val-Ala-Asp-FMK.

7. A pharmaceutical composition according to Claim 6 wherein  
said nitrogen-containing bisphosphonate is alendronate and pharmaceutically  
35 acceptable salts thereof.

8. A pharmaceutical composition according to Claim 7 wherein said nitrogen-containing bisphosphonate is alendronate monosodium trihydrate.
- 5 9. A pharmaceutical composition comprising from about 1 to about 100 mg of a nitrogen-containing bisphosphonate or a pharmaceutically-acceptable salt thereof and from about 0.1 to about 10 mg of a caspase inhibitor.
- 10 10. A pharmaceutical composition comprising from about 2 to about 70 mg of a nitrogen-containing bisphosphonate or a pharmaceutically-acceptable salt thereof and from about 0.1 to about 10 mg of a caspase inhibitor.
- 15 11. A pharmaceutical composition comprising from about 1 to about 100 mg of alendronate monosodium trihydrate, on an alendronic acid weight basis, and from about 0.1 to about 10 mg of a caspase inhibitor selected from the group consisting of group consisting of Z-Val-Ala-Asp-FMK, Z-Asp-Glu-Val-Asp-FMK, Z-Tyr-Val-Ala-Asp-FMK, and mixtures thereof.
- 20 12. A pharmaceutical composition comprising from about 2 to about 70 mg of alendronate monosodium trihydrate, on an alendronic acid weight basis, and from about 0.1 to about 10 mg of a caspase inhibitor selected from the group consisting of Z-Val-Ala-Asp-FMK, Z-Asp-Glu-Val-Asp-FMK, Z-Tyr-Val-Ala-Asp-FMK, and mixtures thereof.
- 25 13. A pharmaceutical composition which is prepared by combining a nitrogen-containing bisphosphonate and a caspase inhibitor.
- 30 14. A method for inhibiting bone resorption in a mammal in need thereof comprising administering a nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof and a caspase inhibitor.
15. A method according to Claim 14 wherein said mammal is a human.

16. An oral method for treating or preventing osteoporosis or Paget's disease in a mammal in need thereof comprising administering a nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof and a caspase inhibitor.
- 5 17. A method according to Claim 16 wherein said mammal is a human.
- 10 18. A method according to Claim 17 wherein said nitrogen-containing bisphosphonate or pharmaceutically-acceptable salt thereof is selected from the group consisting of alendronate, cimadronate, ibandronate, risedronate, piridronate, pamidronate, zolendronate, pharmaceutically acceptable salts thereof, and mixtures thereof.
- 15 19. A method according to Claim 17 wherein said caspase inhibitor is selected from the group consisting of Z-Val-Ala-Asp-FMK, Z-Asp-Glu-Val-Asp-FMK, Z-Tyr-Val-Ala-Asp-FMK, and mixtures thereof.
- 20 20. A method for inhibiting bone resorption in a mammal in need thereof comprising sequentially administering a caspase inhibitor and a nitrogen-containing bisphosphonate or pharmaceutically acceptable salt thereof.

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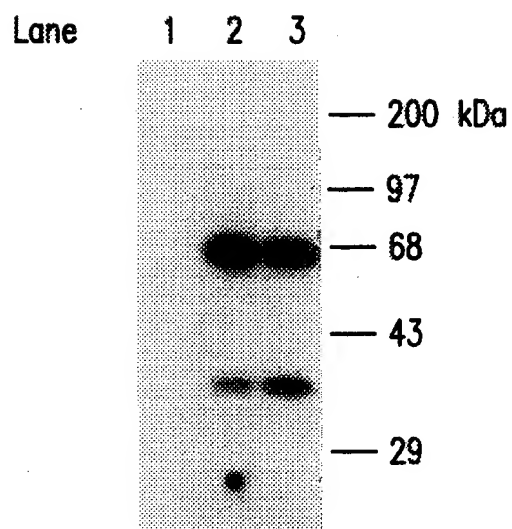


FIG. 1

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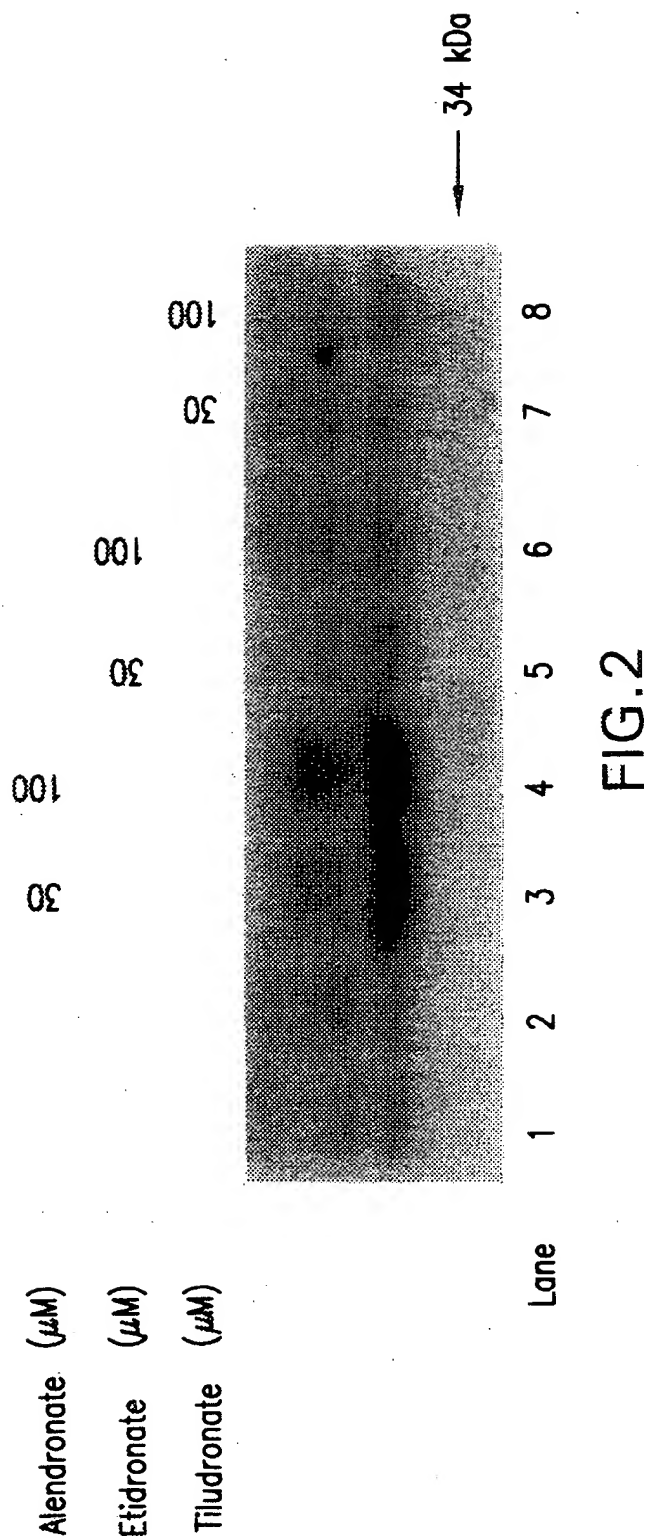


FIG. 2

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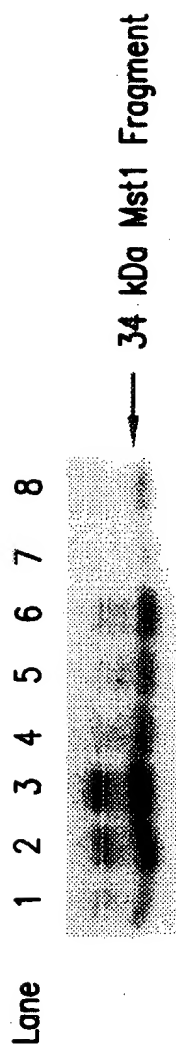


FIG.3

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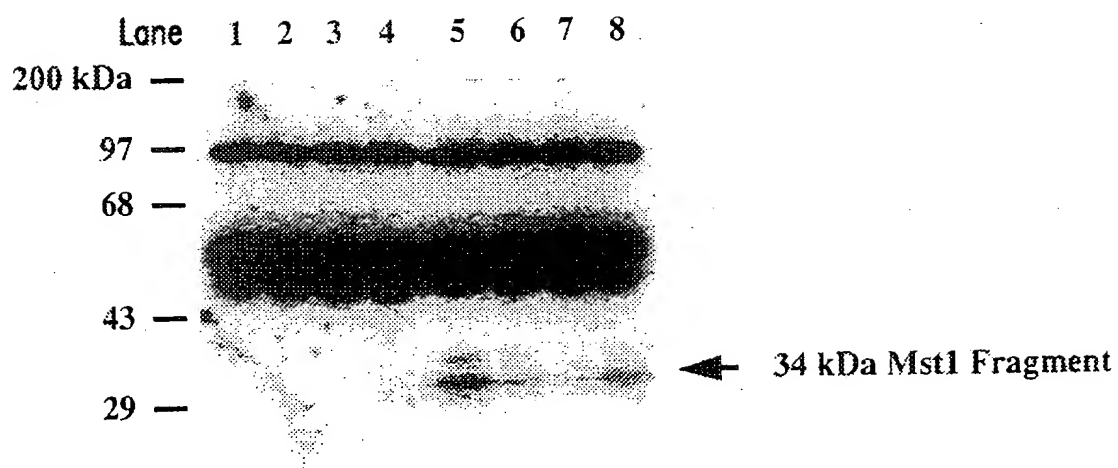


FIG.4



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/13961

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 514/2, 18, 108; 530/330

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2, 18, 108; 530/330

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE, EMBASE, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	COXON, F.P. et al., "Protein Syntheses Is Required For Caspase Activation And Induction of Apoptosis By Bisphosphonate Drugs", Amer. Soc. Pharm. & Exper. Ther., Oct. 1998, Vol. 54 pages 631-8, see entire article.	1-20
A	TAN P.L. et al., "Aminobisphosphonates Inhibition Of Interleukin-1-induce Bone Resorption In Mouse Calvarie", Arthr. & Rheum. June 1988, Vol. 31, No. 6 pages 762-768.	1-20
A	NICHOLSON, D.W. et al., "Caspases: Killer Proteases", TIBS, Aug. 1997, Vol. 22 pages 299-306.	1-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 23 AUGUST 1999	Date of mailing of the international search report 19 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BENNETT CELSA Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/13961

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SANDERS, J.L. et al., "Alendronate/Interleukin-1B Cotreatment Increases Interleukin-6 In Bone and UMR-1-6 Cells: Dose Dependence And Relationship To The Antiresorptive Effect of Alendronate", J. Bone & Min. Res. May 1998, Vol. 13, No. 5 pages 786-792.	1-20
A	HUGHES, D.E. et al., "Bisphosphonates Promote Apoptosis In Murine Osteoclasts In Vitro And In Vivo", J. Bone & Min. Res., 1995 Vol. 10, No. 10 pages 1478-1487.	1-20

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US99/13961

**A. CLASSIFICATION OF SUBJECT MATTER:**

IPC (6):

A61K 38/00, 38/07, 31/66; C07K 5/00

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